and wild-type structures in the vicinity of the active site is shown in Figure 2. The results clearly demonstrate that the Gln side chain forms a bond with the iron, probably via the side-chain oxygen atom,[7] and that all structural changes are localized at the site of mutation.

Enzyme activities were determined by following the oxidation of reduced cytochrome *c* at pH 5.5 (0.1 M Tris-MES-acetate buffer) using excess horse heart cytochrome *c* (40 μM) and varying concentrations of H_2O_2. Kinetic constants were estimated from Eadie–Hofstee plots. For the wild-type enzyme, $k_{cat} = 1463$ s^{-1} and $K_M = 6.7$ μM, and for the Gln mutant, $k_{cat} = 1585$ s^{-1} and $K_m = 58$ μM. That it is possible to drastically change the coordination environment of the heme yet retain nearly full enzyme activity as estimated from the steady-state k_{cat} values is unexpected and calls into question the proposed roles of the proximal ligand. These results demonstrate that the precise nature of the proximal ligand is not critical in the rate-limiting step of the reaction, which very likely is electron transfer from cytochrome *c*.[8,9]

Acknowledgment. We thank Dr. Kamel Darwish for development of the CCP expression system and Dr. Matthew Mauro for invaluable insights on purification and analysis of mutant enzymes. This work was supported by NSF Grant DMB 8716316 and NIH Grant GM 42614.

Registry No. CCP, 9029-53-2; H_2O_2, 7722-84-1; His, 71-00-1; Gln, 56-85-9; Heme, 14875-96-8.

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(7) We cannot be certain that the side-chain oxygen and not the side-chain nitrogen coordinates with the iron although an oxygen–iron bond seems more reasonable since formation of a nitrogen–iron bond would require a deprotonation of the amide nitrogen. Moreover, the 2.9-Å distance between the side chains of Gln175 and Asp235 would favor the amide nitrogen of Gln235 as a hydrogen-bond donor.

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Oxidative Conversion of a Mn(μ-OH)$_2$Mn to a Mn(μ-O)$_2$Mn Moiety. Synthesis and Molecular Structures of a (μ-Hydroxo)dimanganese(II,II) and (μ-Oxo)dimanganese(III,III) Complex with a Hindered N$_3$ Ligand

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Received February 1, 1991

A polymanganese site at the photosystem II oxygen-evolving center (PSII OEC) is known to be responsible for water oxidation to dioxygen. Despite current extensive studies, there is no consensus about the precise arrangement of the manganese atoms, their oxidation states, and the reaction mechanism.[1,2] Because the conversion of water to dioxygen is a four-electron oxidation with release of four protons, the oxidation is formally explicable in terms of the sequential transformation of two water molecules on the polymanganese site: $2H_2O \rightarrow 2OH^- \rightarrow 2O^{2-} \rightarrow O_2^{2-} \rightarrow O_2$. The proposed mechanisms[3,4] for OEC catalysis involve these elemental reaction steps, whereas they are based on a tetranuclear manganese structure for PSII OEC. Very recently, the first example of a (μ-peroxo)dimanganese complex was reported, and it was shown to release dioxygen.[5] This provides a solid chemical basis for the final step ($O_2^{2-} \rightarrow O_2$) in the hypothetical O_2 evolution mechanism. However, neither the oxidative conversion of a bis(μ-hydroxo) to a bis(μ-oxo) moiety nor O–O bond formation from a bis(μ-oxo) moiety on a polymanganese center has been demonstrated to date.

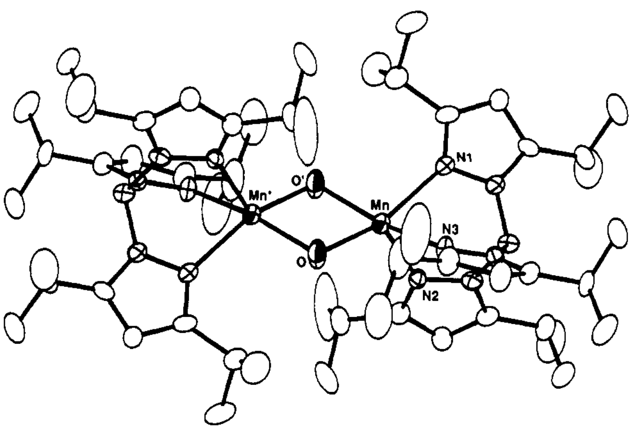

Figure 1. ORTEP view of [Mn(HB(3,5-iPr$_2$pz)$_3$)]$_2$(OH)$_2$ (**2**). The solvents of crystallization are omitted for clarity. Selected bond distances (Å) and angles (deg): Mn–O, 2.094 (4); Mn–O', 2.089 (5); Mn–N1, 2.273 (5); Mn–N2, 2.204 (5); Mn–N3, 2.288 (5); Mn⋯Mn', 3.314 (1); O⋯O', 2.553 (7); Mn–O–Mn', 104.8 (2).

A bis(μ-hydroxo) dinuclear Mn(II,II) complex was successfully prepared with a hindered tris(pyrazolyl)borate ligand in a manner similar to that applied for the synthesis of a bis(μ-hydroxo)copper(II,II) complex, [Cu(HB(3,5-iPr$_2$pz)$_3$)]$_2$(OH)$_2$.[6] The reaction of $MnCl_2$ with 1 equiv of KHB(3,5-iPr$_2$pz)$_3$ in the presence of excess (ca. 5 equiv) 3,5-iPr$_2$pz in a CH_2Cl_2/CH_3OH mixture afforded Mn(Cl)(3,5-iPr$_2$pz)(HB(3,5-iPr$_2$pz)$_3$) (**1**) as an air-stable, white solid. Treatment of a toluene solution of **1** with 1 N aqueous NaOH afforded [Mn(HB(3,5-iPr$_2$pz)$_3$)]$_2$(OH)$_2$ (**2**) in ca. 60% yield as a microcrystalline colorless solid.[8] Single crystals of **2**·6CH_2Cl_2 were obtained by slow recrystallization from CH_2Cl_2 at −20 °C. As shown in Figure 1, **2** has a dinuclear structure that sits on a crystallographically imposed center of symmetry.[9] Two hydroxo groups lie between the two manganese(II) ions with identical bond lengths (2.094 (4) and 2.089 (5) Å). Thus **2** is the first example of a dinuclear manganese complex in which the two manganese ions are bridged solely by hydroxo groups.[10] Previously, one example of a bis(μ-hydroxo)manganese(III,III) complex was reported.[11] However, the Mn–Mn separation (2.72

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(7) Abbreviations: HB(3,5-iPr$_2$pz)$_3$, hydrotris(3,5-diisopropyl-1-pyrazolyl)borate; 3,5-iPr$_2$pz, 3,5-diisopropylpyrazole. Anal. Calcd for $C_{36}H_{61}N_8BMnCl$: C, 61.00; H, 8.61; N, 15.81; Cl, 5.00. Found: C, 60.88; H, 8.99; N, 15.61; Cl, 5.23. IR: ν(NH), 3292; ν(BH), 2528. The structure was established by X-ray crystallography, indicating a very distorted coordination geometry with a N_4Cl ligand donor set. The details will be described elsewhere.

(8) A satisfactory elemental analysis was obtained for **2** dried under vacuum. Anal. Calcd for $C_{54}H_{94}N_{12}O_2B_2Mn_2$: C, 60.03; H, 8.75; N, 15.65. Found: C, 60.42; H, 8.95; N, 15.54. IR: ν(OH) 3688; ν(BH) 2543 cm^{-1}.

(9) **2**·6CH_2Cl_2 ($C_{60}H_{106}N_{12}O_2B_2Cl_{12}Mn_2$; FW 1584.51) crystallized in the monoclinic space group *C*2/*c* with $a = 22.132$ (4) Å, $b = 13.386$ (4) Å, $c = 29.352$ (7) Å, $\beta = 110.56$ (3)°, $V = 8131$ (3) Å^3, and $Z = 4$. Data collection ($2° < 2\theta < 45°$) was made at −50 °C to prevent the loss of CH_2Cl_2 molecules of crystallization. The refinement based on 2950 reflections ($F_o \geq 3\sigma F_o$) converged to final $R(R_w)$ values of 8.01% (7.73%).

(10) A dimanganese(II,II) complex having a Mn(OH)(OAc)$_2$Mn structure was reported: Wieghart, K.; Bossek, U.; Bonvoisin, J.; Beauvillain, P.; Girerd, J.-J.; Nuber, B.; Weiss, J.; Heinze, J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1030.

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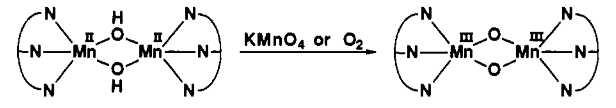
Scheme I



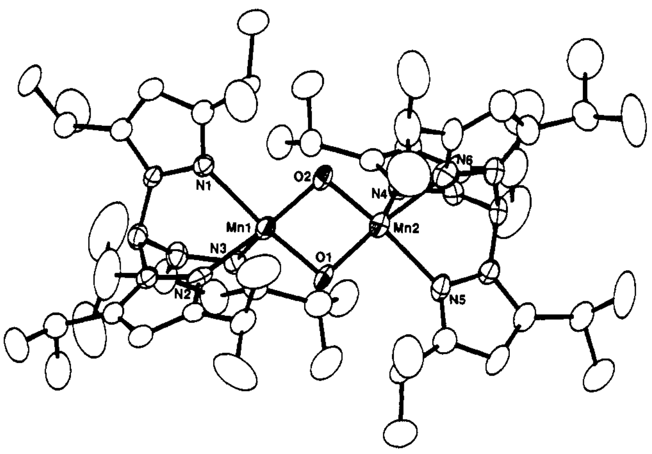


Figure 2. ORTEP view of $[Mn(HB(3,5\text{-}iPr_2pz)_3)]_2(O)_2$ (3). The solvent of crystallization is omitted for clarity. Selected bond distances (Å) and angles (deg): Mn1–O1, 1.806 (5); Mn1–O2, 1.813 (6); Mn1–N1, 2.084 (6); Mn1–N2, 2.099 (7); Mn1–N3, 2.228 (7); Mn2–O1, 1.808 (6); Mn2–O2, 1.787 (6); Mn2–N4, 2.224 (6); Mn2–N5, 2.093 (7); Mn2–N6, 2.084 (7); Mn1···Mn2, 2.696 (2); O1···O2, 2.369 (8); Mn1–O1–Mn2, 96.5 (3); Mn1–O2–Mn2, 97.0 (3).

Å) is too short for the structure and there is some debate as to whether this might be a bis(μ-oxo)manganese(IV,IV) complex. The Mn–Mn separation of **2** (3.31 Å) is much longer than those reported for bis(μ-oxo) dinuclear manganese complexes (ca. 2.7 Å), and it is of interest that the value is close to a Mn–Mn separation known for PSII OEC. EXAFS studies on OEC showed two distinct Mn–Mn separations of 2.7 and 3.3 Å.[12] The shorter distance is associated with a bis(μ-oxo) moiety; however, the structural implications of the longer separation have not yet been unambiguously determined. The possibility of a μ-oxo bis(carboxylato) core structure was pointed out.[1c] On the basis of the XANES result, it is unlikely that the S_1 state of OEC contains two Mn(II) ions.[12] However, because the Mn–Mn separation primarily depends on the core structure rather than the oxidation state, the present result raises the new possibility that the 3.3-Å separation of OEC may be ascribed to a bis(μ-hydroxo) structure. The magnetic susceptibility of the powdered sample of **2** is 6.88 μ_B/mol at 298 K, indicative of a magnetically weak interaction between the two high-spin manganese(II) ions. This is further supported by the X-band EPR spectrum of **2** measured at 77 K, which showed very complicated features as known for magnetically weakly coupled dinuclear Mn(II,II) complexes.[13]

The anaerobic oxidation of **2** with $KMnO_4$ gave a bis(μ-oxo)manganese(III,III) complex (**3**) (Scheme I). In a typical experiment, the colorless solution of **2** was stirred with 4 equiv of $KMnO_4$ for 2 days in toluene. Unreacted $KMnO_4$ was removed by filtration, and the dark-red filtrate was evacuated to dryness under vacuum. Recrystallization of the resultant solid from pentane afforded $\mathbf{3}\cdot C_5H_{12}$ as dark red crystals in ca. 90% yield.[14] The molecular structure of **3** is presented in Figure 2.[15] There is no crystallographically imposed center of symmetry. The Mn–O distances are in the range 1.79–1.81 Å, which are typical Mn–O bond distances for manganese–oxo bonds. The Mn–Mn separation of 2.70 Å is also typical for the dinuclear complexes having a $Mn(\mu\text{-}O)_2Mn$ moiety. There are many examples of bis(μ-oxo)dimanganese complexes of Mn(III,IV) and Mn(IV,IV), whereas Mn(III,III) complexes were not known until very recently.[16] All these complexes contain six-coordinate manganese ions. On the other hand, the manganese ions in **3** are five-coordinate. This is attributable to the large steric hindrance of the present ligand. Complex **3** is EPR silent and gives a reasonably sharp ^{1}H NMR spectrum at low temperature. These facts together with the low magnetic susceptibility (2.81 μ_B/mol) suggest a strong antiferromagnetic interaction between the two manganese(III) ions in **3** as observed for other bis(μ-oxo)manganese(III) complexes,[16] although the coordination geometry is not comparable.

Complex **2** is also oxidatively converted to **3** under a dioxygen atmosphere. However, the yield to **3** was not very high (the highest yield was ca. 50%), and we noticed that at least two other manganese complexes are formed. Isolation and characterization of these species as well as further oxidation of **3** in the hope of evolving dioxygen are proceeding in this laboratory.

Acknowledgment. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (02750589). We appreciate the kind editing of this manuscript by Professor L. Que, Jr., of University of Minnesota. The kind help in the X-ray analysis of $\mathbf{3}\cdot C_5H_{12}$ by Dr. M. Tanaka in this institute is acknowledged.

Supplementary Material Available: Summary of X-ray analysis, atomic coordinates, anisotropic thermal parameters, and bond distances and angles for $\mathbf{2}\cdot 6CH_2Cl_2$ and $\mathbf{3}\cdot C_5H_{12}$ (24 pages); listing of observed and calculated structures factors for $\mathbf{2}\cdot 6CH_2Cl_2$ and $\mathbf{3}\cdot C_5H_{12}$ (45 pages). Ordering information is given on any current masthead page.

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(14) Satisfactory elemental analysis was obtained for **3** dried under vacuum. Anal. Calcd for $C_{54}H_{92}N_{12}O_2B_2Mn_2$: C, 60.39; H, 8.57; N, 15.65. Found: C, 60.09; H, 8.74; N, 15.80. IR: ν(BH), 2535 cm^{-1}. UV–vis [λ_{max}, nm (ϵ, cm^{-1} M^{-1})] (toluene): 468 nm (315). ^{1}H NMR (toluene-d_8, –40 °C): 1.43 (br, 72 H, Me_2CH), 5.75 (s, 6 H, Me_2CH), 6.95 (s, 6 H, Me_2CH), 8.96 (s, 6 H, pz).

(15) $\mathbf{3}\cdot C_5H_{12}$ ($C_{59}H_{104}N_{12}O_2B_2Mn_2$; FW 1145.05) crystallized in the monoclinic space group $C2/c$ with $a = 48.325$ (9) Å, $b = 15.747$ (6) Å, $c = 18.596$ (2) Å, $\beta = 106.21$ (3)°, $V = 13\,588$ (2) Å^3, and $Z = 8$. $D_{calcd} = 1.12$; $D_{measd} = 1.12 \pm 0.01$ g cm^{-3}. The current R and R_w factors are 8.83% and 10.53% for 5420 reflections ($5° < 2\theta < 45°$, $F_o \geq 3\sigma F_o$).

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Gold Cluster Laden Polydiacetylenes: Novel Materials for Nonlinear Optics

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Received May 20, 1991

Since the early work of Maxwell-Garnett,[1] there has been an interest in the optical properties of metal clusters embedded in a dielectric medium. Many recent studies have been reported on the dispersion of metal clusters in organic polymers.[2] We are interested in embedding metal clusters in a special class of organic polymers, namely, nonlinear optical (NLO) polymers. We describe here the synthesis and characterization of gold cluster laden polydiacetylenes. Separately, gold clusters[3] and polydiacetylenes[4]

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